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EVALUATION OF THE EFFECT OF ANTICOCCIDIAL DRUGS ON SIZE OF PARASITE POPULATION AND ITS DEVELOPMENT IN LITTERS

Muriel NACIRI 1, P. YVORÉ 1 and L. CONAN 2

1 INRA, CR de Tours-Nouzilly, Station de Pathologie Aviaire et de Parasitologie, 37380 Monnaie, France
2 INRA, Domaine expérimental du Magneraud, Saint-Pierre-d’Amilly, BP 52, 17700 Surgères, France

Résumé
ÉVALUATION DE L’ACTION DES ANTICOCCIDIENS SUR L’IMPORTANCE DE LA POPULATION PARASITAIRE ET SON ÉVOLUTION DANS LES LITIÈRES. — L’évaluation du nombre d’oocystes présents dans les litières et de leur taux de sporulation est assez facile à réaliser en floor pens. Nous avons pu constater que cette contamination varie considérablement suivant l’anticoccidien présent dans l’aliment. Malgré une différence d’action sur le développement parasitaire, on constate des performances identiques dans tous les lots traités. Le Monensin est parmi les anticoccidiens étudiés, celui qui réduit le moins la contamination du milieu ; l’Halofuginone est celui qui la réduit le plus.

Presence of coccidia in industrial poultry breeding is accepted to be inevitable. Current high density production techniques on permanent litters favour contamination of environment and increase risk of parasitic infection all the more because such conditions may modify sensitivity of animals by increasing breeding stress.

Several methods for evaluating risk of contamination of animals have been proposed: evaluation of the number of oocysts present in the litter; study of oocyst excretion of breeding animals at a given time; introduction of sensitive animals and investigation of their level of contamination after a period spent in breeding (sentinel birds). We have chosen here to study contamination of litters by evaluating the number of oocysts present and their rate of sporulation in relation to the anticoccidial drug used and the time of sampling. We carried out this study in floor pens by observing the development of litter contamination and progress in performances of animals.

Materials and Methods
1. Experimental design
The study was carried out in the form of two experiments conducted in floor pens with contaminated feed (Yvoré et al., 1980). Sexed animals were split up into 6 m² pens with wood shaving litters at a density of 14 per m² in the first experiment and 25 per m² in the second. Sexes were segregated.
Birds were fed ad libitum with a « starter » feed up to day 28 and a « grower » feed from day 29 to the end of the experiment (day 45 or day 49). They received contaminated feed on four consecutive days. Chickens were 13 days old at the start of infection. They received on average 15 000 *Eimeria tenella* oocysts and 100 000 *E. acervulina* oocysts per day.

In the first experiment there were four groups, all infected, each consisting of six pens (3 male, 3 female). Groups were as follows : the first group is non treated; the second is treated with 100 mg/kg of Monensin, the third with 3 mg/kg of Halofuginone and the fourth with 160 mg/kg of Pancoxin plus.

In the second experiment there were five groups, each consisting of four pens (2 male, 2 female). Groups were as follows : the first group is infected non treated, the second is treated with 100 mg/kg of Monensin, the third with 3 mg/kg of Halofuginone, the fourth with 60 mg/kg of Salinomycine and the fifth is treated with 60 mg/kg of Salinomycine in « starter » feed, and after with 3 mg/kg of Halofuginone in « grower » feed (shuttle program).

Anticoccidial drugs were administered continually in feed throughout the whole breeding period. The inoculum was mixed for each group with feeds corresponding to that group.

2. Measurements

2.1. Performances

Birds were weighed individually at 1, 10, 28 and 45 or 49 days of age ; feed consumption per pen was measured and feed conversion indices calculated.

2.2. Contamination of litters

In each case three samples were taken from three clearly-defined areas (around a feeding dish, around the water dish, under the heating lamp), on each day of measurement. Material was collected from the surface of these litters in plastic bags kept at + 4 °C.

After soaking for 24 h in water at + 4 °C, the sample was filtered and washed on a mesh of 1 000 or 630 μ to eliminate shavings and collect all faeces. Filtrate was centrifuged for 10 min at about 2 500 t/min. Sediment consisted essentially of faeces hydrated in a constant manner. Five grammes of sediment were suspended in 70 ml of Magnesium sulphate solution (d ≃ 1.25). Counting was done on a Mac Master slide. Value was expressed by the number of oocysts per gramme of sediment (hydrated faeces).

After concentration by floating the rate of sporulation was evaluated by examination under the microscope of 200 oocysts per sample.

2.3. Analysis

Analysis of variance (Fisher test) and comparison of means (Duncan test) were used for all criteria studied.

Results

1. Performance of animals

During the period following infection (day 10 to day 28), we note in both experiments that parasitism has diminished growth significantly (- 11 % in the first experiment and - 20 % in the second) and increased the feed conversion index (table 1). If the breeding period as a whole is considered, differences between the groups are smaller, but it is still the infected, non-treated group which gives the poorer performances : average weight of animals is lower, conversion index higher. Practically all anticoccidial drugs gave the same results : effect of parasitism was reduced and performances were apparently normal. In the first experiment, however, there is a rather low weight gain for a normal index in the group receiving Monensin, and in the second experiment performances are slightly poorer in the group treated with Halofuginone. Between day 10 and day 28, however, control of parasitism is the same for all anticoccidial drugs and differences observed in the second experiment for the total period are only slightly significant (P < 0.05).

2. Contamination of litters

For technical reasons, the first sample after infection of animals was not taken on the same date in the two experiments. In both cases, however, there are very significant differences between the groups (table 1). Reduction of number of oocysts is very high in groups treated with Halofuginone. It is lower in groups treated with Salinomycine (Salinomycine 60 ppm group and shuttle program group which at this time had received this anticoccidial drug only) and with Pancoxin plus. Finally, in the group treated with Monensin, level of contamination remains high, especially in the first experiment. Effect on the number of oocysts sporulated varies with the anticoccidial drug in question. Whilst for Halofuginone there is apparently no
reduction in the only case where this could be measured, there is a reduction of 30-50 % for Monensin and Salinomycine and over 90 % in the group treated with Pancoxin plus.

At the end of the breeding period contamination of litters has diminished in all groups. The same differences between groups persist. Finally, percentage of oocysts sporulated remain more or less the same from the first measurement.

**Table 1.** - Average weight gain and feed conversion index from Day 10 to Day 28 (initial contamination period) and from Day 1 to the end of experiment (total period of breeding)

<table>
<thead>
<tr>
<th>Weight gain (g)</th>
<th>Feed conversion (F/G)</th>
<th>Weight gain (g)</th>
<th>Feed conversion (F/G)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial contamination period</strong></td>
<td></td>
<td><strong>Total period</strong></td>
<td></td>
</tr>
<tr>
<td>(Day 10-28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monensin (100 ppm)</td>
<td>644 A</td>
<td>1.57 B</td>
<td>1679 B</td>
</tr>
<tr>
<td>Halofuginone (3 ppm)</td>
<td>681 A</td>
<td>1.52 B</td>
<td>1724 A</td>
</tr>
<tr>
<td>Pancoxin plus (160 ppm)</td>
<td>667 A</td>
<td>1.56 B</td>
<td>1726 A</td>
</tr>
<tr>
<td><strong>Second experiment : 25 per m²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non treated</td>
<td>535 B</td>
<td>2.06 A</td>
<td>1589 b</td>
</tr>
<tr>
<td>Monensin (100 ppm)</td>
<td>649 A</td>
<td>1.78 B</td>
<td>1711 a</td>
</tr>
<tr>
<td>Halofuginone (3 ppm)</td>
<td>658 A</td>
<td>1.78 B</td>
<td>1657 ab</td>
</tr>
<tr>
<td>Salinomycine (60 ppm)</td>
<td>672 A</td>
<td>1.70 B</td>
<td>1735 a</td>
</tr>
<tr>
<td>Shuttle program</td>
<td>654 A</td>
<td>1.71 B</td>
<td>1670 a</td>
</tr>
</tbody>
</table>

a : average per group ; for each column, any means with different superscript are different \( P < 0.01 \) (A, B, C); \( P < 0.05 \) (a, b, c, d).

**Table 2.** - Average number of oocysts per gramme of litter and per cent sporulated per group at different time of breeding

<table>
<thead>
<tr>
<th>Days after the start of experiment</th>
<th>D 22</th>
<th>D 28</th>
<th>D 48</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First experiment : 14 per m²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non treated</td>
<td>...</td>
<td>227 800 A (49)a</td>
<td>113 800 A (44)</td>
</tr>
<tr>
<td>Monensin (100 ppm)</td>
<td>...</td>
<td>118 500 A (25)</td>
<td>18 400 B (31)</td>
</tr>
<tr>
<td>Halofuginone (3 ppm)</td>
<td>...</td>
<td>380 C</td>
<td>110 D</td>
</tr>
<tr>
<td>Pancoxin plus (160 ppm)</td>
<td>...</td>
<td>34 300 B (4)</td>
<td>6 300 C (3)</td>
</tr>
<tr>
<td><strong>Second experiment : 25 per m²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non treated</td>
<td>882 000 A (48)</td>
<td>...</td>
<td>72 800 A (50)</td>
</tr>
<tr>
<td>Monensin (100 ppm)</td>
<td>90 600 B (34)</td>
<td>...</td>
<td>8 600 B (30)</td>
</tr>
<tr>
<td>Halofuginone (3 ppm)</td>
<td>1 570 D (53)</td>
<td>...</td>
<td>190 D</td>
</tr>
<tr>
<td>Salinomycine (60 ppm)</td>
<td>10 300 C (34)</td>
<td>...</td>
<td>1 030 C (26)</td>
</tr>
<tr>
<td>Shuttle program</td>
<td>12 200 C (34)</td>
<td>...</td>
<td>1 630 C (28)</td>
</tr>
</tbody>
</table>

a : oocysts per gramme of « hydrated fecal matter »; for each column and the same experiment any means with the same superscript are not different at \( P < 0.01 \); in brackets : P. cent of sporulated oocysts.
Discussion

Technique used proved satisfactory. Inter-group and even inter-pen variations are quite wide but enable us, however, to see significant inter-group differences, given the extent of these differences. Measurement of rate of sporulation is easy if contamination of litter is sufficiently high. In the case of groups treated with Halofuginone reduction in development was such that we were only able to take this measurement in one case.

Evaluation of contamination of litters has already given rise to a certain number of studies. Horton-Smith and Long (1954), and Reid and Raja (1963), have attempted to evaluate the number of coccidial oocysts contained in litters. Long and Rowell (1975), and Long and Millard (1977), have proposed a suitable technique for estimating this number. The one we used in our study is very similar to theirs. They have noted that the number of oocysts varies with time, reaching a maximum between the fourth and sixth week.

Other authors recommend examination not of litters but of freshly excreted faeces (Hodgson, 1970; Hamet et al., 1981). As Long and Rowell (1975) point out, this method produces an index of the infection of birds themselves rather than an evaluation of the number of oocysts present in the breeding environment.

The fact remains that these evaluations give an idea of the number of oocysts present but not of their pathogenicity. Long et al. (1980), have proposed a very interesting method for determining the latter. They introduce new, sensitive birds into the breeding area for a short period (48 h), and then evaluate their degree of infection in the pen. These sentinel birds could enable indirect evaluation of pathogenicity of the environment, and this technique seems more sensitive than the counting of oocysts. It may possibly, however, have two disadvantages. Firstly, behaviour of new animals introduced into the group from outside may be very particular, secondly, their introduction into anticoccidial floor pen trials could disturb the experimental design. This is why we preferred to investigate oocysts and the rate of sporulation of the population at the same time. If this rate does not take into consideration the pathogenicity of the parasitic element, it can still give an account of its state of maturity and its capacity to develop.

It can be observed that parasite population in litters differs widely according to groups. Halo-

Pancoxin plus, in view of its action on sporogony, also reduces considerably the number of sporulated oocysts (1.2 % of control group). Lowest reduction is observed in the group treated with Monensin; furthermore it is fairly different in the two experiments. If Joyner and Norton (1977), have noted the effect of Amprolium, a constituent part of Pancoxin plus, on sporogony, they have observed no effect at this level in the group receiving Monensin. Our results, appearing to contradict this, may arise from a different activity on the two species of coccidia used. In general, rate of sporulation varies with the species; Monensin would less inhibit development of the species with the lowest rate, resulting in an average rate in the litter population lower than that of the control group. We have been unable to prove this hypothesis, which does however seem the most likely.

Even if one observes, as Long and Rowell (1975), and Long and Millard (1977) have done, a reduction in the number of oocysts in all groups at the end of the breeding period, differences between groups persist. Furthermore, contrary to what one may think, density does not seem to modify the values, which in both experiments are very similar at the end of the breeding period.

Finally, under the conditions of our trial, there exists apparently no relation between performances and rate of parasitic development. This is particularly clear in the case of Monensin, where, despite a limited reduction in oocyst output, performances may be considered normal.

Conclusion

Reid (1975, 1978) doubted the interest of using an « oocyst excretion » criterion in the study of the efficiency of anticoccidial drugs. On condition that rate of sporulation, as a result of the effect of a certain number of anticoccidial drugs on sporogony, is also considered, this epidemiological criterion seems to us to be of interest. It is fairly easy to measure and gives an idea of contamination of environment. Even if it is only of minor interest in judging efficiency of the
Anticoccidial drug in protection of animals, it allows evaluation of the risks of contamination in future groups, particularly in the case of permanent litters. We have been able to observe (Yvoré et al., 1980), that the effectiveness of an anticoccidial drug may differ according to the extent of contamination. A group receiving an anticoccidial drug, contaminated to a low degree and only accidentally, gave performances superior to those of another group treated with the same anticoccidial drug and infected experimentally with a large dose of oocysts. It is therefore of interest, for the results of future groups, to know the effect of a product on the size of parasitic population and on its development in the litter.

It will be in the interest of future users of the permanent litter method to achieve lowest possible levels of contamination.

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Summary

Evaluation of the number of oocysts present in litters and of their rate of sporulation is relatively easy in floor pens. We have observed that contamination varies considerably according to the anticoccidial drug present in feed. Despite a difference in effect on parasitic development, some identical performances were noted in all groups treated. Among the anticoccidial drugs studied, Monensin is the one which least reduces contamination of environment; Halofuginone is the one which reduces contamination the most.

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HORTON-SMITH C., LONG P.J., 1954. Preliminary observations on the physical conditions of built up litter and their possible effects on the parasite populations. 10th World's Poul. Congr., Edinburgh, 266-273.


